size) were determined by SEM. For cell culture studies, 5mm explants punched from bovine articular cartilage were wounded with a scalpel and equilibrated in culture for 24h. Explants were subsequently incubated with 0 or 10ng/mL of IL-1β and/or microcapsules with or without encapsulated CNP for 96h. At the end of the culture period, samples were analysed for NO release and GAG loss (%). Chondrocyte % viability was assessed by incubation with calcein-AM and EtBr-1(5μM), and quantified by ImageJ analysis. Confocal microscopy was used to detect interaction of CNP microcapsules by incubating chondrocytes with antibodies to natriuretic peptide receptor (Npr) 2 and 3.
Results: SEM showed uniform, 2-3 μm spherical microcapsules with morphological characteristics similar in templates loaded with or without CNP. The protein was localized around the inner surface of the microcapsule shell with encapsulation efficiencies >82.9%. CNP release profiles were broadly similar following 1-9 days of culture. The presence of CNP microcapsules did not significantly affect cell viability (>80%), with DNA values that remained stable throughout theculture conditions. Microcapsules were shown to localise to wounded areas of the explants, and confocal imaging showed clustering of microcapsules in chondrocytes to natriuretic peptide receptor (Npr) 2 and 3. Furthermore, treatment of cartilage explants with CNP microcapsules led to concentration-dependent inhibition of NO release in response to IL-1βand restoration of matrix synthesis.
Conclusions: The present study reproducibly generated uniform microcapsules with homogeneous morphological characteristic suitable for delivery and retention in the articular joint. The microcapsule delivery system is highly effective for CNP since the peptide could be stabilised through electrostatic interaction with the polyelectrolyte layers sufficient to allow diffusion of active CNP after localisation to areas of damage. Furthermore, treatment of cartilage explants with bioactive concentrations of CNP led to protective effects with sustained release of CNP in a manner responsive to the local environment. In summary, we demonstrate for the first time controlled delivery of CNP to dampen inflammatory effects induced by IL-1βin cartilage explants that has the potential to promote cartilage repair in vivo.

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OBESITY DOES NOT NEGATIVELY INFLUENCE CARTILAGE REPAIR OR CAUSE OSTEOARTHRITIS IN DBA/1 MICE

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Purpose: Traumatic cartilage damage, if left untreated, could eventually increase the risk of developing osteoarthritis (OA). Marrow stimulation treatments, such as microfracture, are performed to stimulate intrinsic cartilage repair. The symptomatic improvements of microfracture are reported to be less in obese patients and these patients are now often excluded from treatment. Obesity is a major risk factor for OA and leads to low grade systemic inflammation and metabolic changes. There is however no convincing evidence that intrinsic cartilage repair is negatively influenced by obesity. To optimize cartilage repair treatments and to prevent OA development in obese patients, it is therefore essential to investigate whether and how obesity influences cartilage repair. In this study we investigated the effects of obesity on cartilage repair and OA development in the DBA/1 mouse strain. Our hypothesis was that obesity negatively influences intrinsic cartilage repair and accelerates the speed of OA development after cartilage damage.
Methods: Ten-weeks-old male DBA/1 mice were fed with control diet or obesity inducing high fat diet (HFD; 60% energy from fat). After two weeks, a full thickness cartilage defect was made in the trochlear groove of the left knee. Mice were sacrificed after 1 (n=6 mice per diet), 8 (n=9 mice per diet) and 24 (n=5 mice per diet) weeks. Cartilage repair was evaluated on histology using the Pineda scoring method and OA development was evaluated using the OARSI scoring method.
Results: Mice on a HFD had higher bodyweight when making the defect (31.0±1.7gr versus 27.2±1.1gr; p<0.001) and the difference became even larger after 24 weeks (42.0±5.8gr versus 31.7±4.6gr; p<0.001). One week after defect creation, mice on HFD had a higher percentage of defect filling with fibroblast-like cells in the defect. After 8 weeks, mice on a HFD had more cartilage repair as indicated by a lower Pineda score (p=0.01). After 24 weeks, no mice had complete cartilage repair and we were not able to detect a statistically significant difference in Pineda score between mice on HFD and control diet with this group size. All mice at every time point had an OARSI score of 0, indicating no signs of osteoarthritis.
Conclusions: Obesity did not negatively affect intrinsic cartilage repair in DBA/1 mice. Obesity did not cause OA in DBA/1 mice, even in combination with induced traumatic cartilage damage. Future research into the inflammatory and metabolic changes after a high fat diet in this strain of mice could provide more insights into the link between obesity related changes, cartilage damage and OA development.

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CARTILAGE TISSUE ENGINEERING USING ADAMTS-4/5 DEFICIENT MESENCHYMAL STEM CELLS

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Purpose: Aggrecan is one of the two major constituents of articular cartilage, and during diseases such as osteoarthritis (OA) it is subject to degradation by proteolytic enzymes. The primary proteases responsible for aggrecan cleavage are the aggrecanases, identified as members of the disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family of proteases. Aggrecanase 1(ADAMTS-4) and aggrecanase 2 (ADAMTS-5) have been reported as important therapeutic targets in osteoarthritis. However, there is still debate which of them is the major aggrecanase responsible for the degradation of aggrecan in human cartilage. The aim of this study was to generate ADAMTS-4 deficient and ADAMTS-5 deficient chondroprogenitors derived form human induced pluripotent stem cells (iPSCs) to enable the evaluation of the respective roles of ADAMTS-4 and ADAMTS-5 in human cartilage breakdown.
Methods: ADAMTS-4 deficient and ADAMTS-5 deficient human iPS cells were produced by knockout of these genes in iPS cells using Transcription Activation-Like Effector nucleases (TALENs). These iPS cells were differentiated into chondrogenic lineage via generation of mesenchymal stem cells (MSCs) using a multistep culture method consisting of embryoid body (EB) formation, cell outgrowth from EBs, monolayer culture of sprouted cells from EBs, and 3-demensional pellet culture. Interleukin-1 (IL-1) and tumor necrosis factor (TNF) treatment were applied after chondrogenic differentiation. The expression of type II collagen, aggrecan, ADAMTS4, and ADAMTS-5 was measured by quantitative real-time PCR, Western blotting, and immunofluorescence staining. The production of collagen and glycosaminoglycan (GAG) was quantified by dye binding assays.
Results: ADAMTS-4/5 deficient iPSC-derived MSCs exhibited fibroblast-like morphology similar to bone marrow MSCs and expressed surface markers for MSCs. After 4-6 weeks of pellet culture, cells in pellet exhibited a spherical morphology typical of chondrocytes. The expression of type II collagen and aggrecan in pellets progressively increased. Histological analysis revealed that ADAMTS-4/5 deficient MSC-derived pellets successfully underwent chondrogenic differentiation.
Conclusions: These results demonstrate a model system of chondroprogenitors from genetically modified human iPSCs. This work also provides potential iPSC progeny for developing cell-base approaches to repair joint cartilage damage.

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EXTRACELLULAR MATRIX CHANGES IN RESPONSE TO SPRIFERMIN STUDIED IN EX VIVO CULTURES OF ARTICULAR CARTILAGE

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Purpose: Osteoarthritis (OA) is a degenerative disease with high prevalence, creating an unmet medical need for drugs to protect and regenerate cartilage. Sprifermin, a truncated form of fibroblast growth factor 18 (rhFGF18), is being investigated as a potential disease-modifying OA drug (DMOAD). Sprifermin has been shown to increase cartilage volume in the knees of OA patients. The few studies published about the mode of action behind its anabolic effects, indicate that full-